EXPERT REVIEWS

Fungal central nervous system infections: prevalence and diagnosis

Expert Rev. Anti Infect. Ther. Early online, 1–9 (2014)

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Fungal infections of the central nervous system (CNS) are rare but they pose a significant challenge. Their prevalence spans a wide array of hosts including immunosuppressed and immunocompetent individuals, patients undergoing neurosurgical procedures and those carrying implantable CNS devices. *Cryptococcus neoformans* and *Aspergillus* spp. remain the most common pathogens. Magnetic resonance imaging can help localize the lesions, but diagnosis is challenging since invasive procedures may be needed for the retrieval of tissue, especially in cases of fungal abscesses. Antigen and antibody tests are available and approved for use in the cerebrospinal fluid (CSF). PCR-based techniques are promising but they are not validated for use in the CSF. This review provides an overview on the differential diagnosis of the fungal CNS disease based on the host and the clinical syndrome and suggests the optimal use of diagnostic techniques. It also summarizes the emergence of *Cryptococcus gatti* and an unanticipated outbreak caused by *Exserohilum rostratum.*

Keywords: abscess • cerebral • fungal • meningitis • molds • yeasts

The incidence of fungal infections of the CNS has been increasing. CNS fungal infections can present as meningitis, meningoencephalitis, brain abscesses or stroke syndrome due to vascular invasion [1,2]. Despite the progress in microbiologic and imaging techniques, their diagnosis remains a challenge [2] and, in a significant percentage of patients, invasive procedures are required for diagnosis and treatment [2].

In this review, we summarize the recent literature on the prevalence of the CNS mycoses and the progress in diagnostic techniques. We focus on the problems resulting from the emergence of new pathogens and the new patient populations that are at increased risk for fungal infection of the CNS. *Cryptococcus* spp. remain the most common cause of fungal meningitis [3]. In the last decade, *Cryptococcus gattii* (a pathogen traditionally isolated in tropical and subtropical areas) has caused meningitis outbreaks in temperate climates [4]. *Aspergillus* spp. remain a major cause of morbidity and mortality in severely immunocompromised individuals, while other molds are becoming more common [5,6]. Finally, in 2012, epidural steroid injections infected with environmental molds were responsible for an outbreak of meningitis that was responsible for about 60 deaths. This outbreak underlined the possible threats when fungi cross from the plant kingdom to humans [7].

Susceptible populations

Different patient populations are at risk for different types of fungal infections of the CNS. In Table 1, we present the most important fungal pathogens that cause CNS disease in certain settings.

In the HIV/AIDS population, cryptococcal meningitis remains the most common fungal infection of the CNS, with a decrease in incidence after the introduction of combination antiretrovital therapy (cART). Unfortunately, this decrease in incidence is, for the most part, limited to the USA and Europe [8]. Moreover, HIV infection predisposes to CNS infections by endemic fungi and *Aspergillus* spp., which have a greater incidence in HIV disease than the general population [1].

Patients with hematologic malignancy and neutropenia are particularly susceptible to CNS aspergillosis, while the emergence of non-*Aspergillus* molds has gained special attention [1,9]. Overall, up to 10% of the mold infections in

 10.1586/14787210.2014.874282 ISSN 1478-7210

Table 1. Main pathogens associated with CNS disease according to predisposing conditions.

Predisposing conditions	Pathogens
HIV/AIDS	*Cryptococcus* spp. Endemic fungi *Aspergillus* spp.
Leukemia/neutropenia	*Aspergillus* spp. Mucorales *Candida* spp.
Hematopoietic stem cell transplants	*Aspergillus* spp. Mucorales *Fusarium* spp.
Solid organ transplants	*Aspergillus* spp. *Candida* spp. *Cryptococcus* spp.
Corticosteroids	*Aspergillus* spp. *Candida* spp. Mucorales
Autoimmune disorders	*Cryptococcus* spp. *Aspergillus* spp. *Candida* spp.
TNF inhibitors use	*Cryptococcus* spp. *Histoplasma capsulatum*
Neurosurgery	*Candida* spp.

the neutropenic population can have CNS involvement [1]. *Candida* spp. CNS infections may also be associated with neutropenia; nevertheless they are less common [1]. Of note is the fact that fungal CNS infections are not common in patients with chronic lymphocytic leukemia [1]. However, the use of certain immunosuppressive therapies like alemtuzumab and fludarabine may make them more prevalent [10–12].

Hematopoietic stem cell transplant patients demonstrate an increased susceptibility to *Aspergillus* spp., both in the neutropenic phase and later during the period of increased immunosuppression [1,9]. Mucormycosis is the second most common fungal CNS infections and some reports indicate that the incidence of mucormycosis in this population is increasing [1,2,6]. Solid organ transplant recipients are also susceptible to CNS infections caused by *Candida* spp., *Cryptococcus* spp., *Aspergillus* spp. and other molds [1,6].

Interestingly, there has been an increased appreciation of the fungal CNS infections in patients with autoimmune disorders and especially systemic lupus erythematosus (SLE). SLE patients are more susceptible to invasive fungal infections than the patients with rheumatoid arthritis (1.04 vs 0.15%) [13], while in neutropenia, the use of high-dose steroids and the cumulative steroid dose also increase the susceptibility to fungal CNS invasion [13,14]. Infection by *Cryptococcus* spp. remains the most common cause of fungal meningitis and cerebral abscesses in this population [2,13], while CNS disease due to *Aspergillus* spp., *Candida* spp. as well as mucormycosis is less common [13–15]. Relatively few cases of fungal CNS infections have been reported in patients on TNF inhibitors, nevertheless *Cryptococcus* spp. and *Histoplasma capsulatum* are the most common offending agents [1,16].

In addition to immunosuppressed individuals, neurosurgical patients are also at risk for fungal infections of the CNS. Although they are rare, *Candida* spp. and *Cryptococcus* spp. infections have been reported as well as ventriculo-peritoneal shunt infections caused by *H. capsulatum* and *Coccidioides immitis* [1,17,18].

Clinical syndromes

In Table 2, we summarize the pathogens implicated in CNS fungal infections categorized as yeasts, molds and endemic fungi and whether they are mostly associated with brain abscesses, meningitis, meningoencephalitis or stroke syndrome.

Knowledge of the local epidemiology and the host susceptibility may guide the differential diagnosis. Clinicians should keep in mind that even among cases of meningitis, in the immunocompromised host the classic meningitis triad of fever, nuchal rigidity and altered mental status may be absent, mainly because of altered inflammatory response [1,19,20]. Brain abscesses usually present with focal neurologic abnormalities and occasionally seizures, whereas angioinvasive disease causes stroke-like syndromes [1].

Diagnosis

The radiologic differentiation of the cerebral mycoses is very difficult because the findings are non-specific [21]. Yeasts usually spread hematogeneously and enter the meningeal microcirculation often resulting in meningitis, while parenchymal disease is more common among CNS infection caused by molds that tend to invade larger vessels resulting in vasculitis and infarcts [21].

Imaging studies

As noted above, parenchymal fungal infection of the CNS usually presents as granuloma, cerebritis and abscess, and is more often associated with aspergillosis and mucormycosis [21,22]. CT imaging is a valuable tool and it is probably the first study to be obtained but it cannot distinguish fungal abscesses [21]. Fungal abscesses are often multiple whereas bacterial abscesses are single, they do not involve the deep gray matter and spare the basal ganglia [21]. However, fungal abscesses may be indistinguishable from pyogenic abscesses with the demonstration of ring-enhancing lesions. Of note is that ring enhancement may be lacking in the case of severe immunosuppression and in the cerebritis stage when the abscess has not yet formed [21]. In cases of cerebral disease that arises from sinusitis, the imaging studies are helpful, especially MRI is more diagnostic since the decreased signal intensity in the T1- and T2-weighted images can differentiate fungal from bacterial disease [21]. MRI is usually the next imaging study to be obtained after an initial CT scanning. In the case of fungal abscesses, MRI can show projections directed into the abscess cavity that do not enhance with contrast [23]. Newer MRI techniques such as MR perfusion, diffusion tensor imaging and diffusion-weighted imaging may

Table 2. Yeasts and molds causing CNS infection and predominant clinical syndromes.

	Cerebral abscess	Meningitis	Meningoencephalitis	Stroke syndrome
Yeasts				
Candida spp.	+++	+	++	+
Cryptococcus spp.	+	++++	++	+
Molds				
Aspergillus spp.	++++	+	+	++
Mucorales	+++	0	+	++
Penicillium marneffei	++	+	+	0
Scedosporium spp.	+++	+	++	++
Phaeohyphomycosis	+++	+	+	0
Exserohilum rostratum	+	++++	++	++
Endemic fungi				
Blastomyces dermatitidis	+++	++	+	0
Histoplasma capsulatum	++	++	+	+
Coccidioides immitis	+	++++	++	+
Paracoccidioides brasiliensis	++++	+	+	0

prove useful in the future [21,24]. Magnetic resonance spectroscopy may identify trehalose, mannitol and lipids, which are elements of the fungal wall and help in the differential diagnosis from tumors [21,23,24].

The need for early differentiation between bacterial and fungal infections has led to the investigation of ^{131}I-labeled with monoclonal antibodies directed against the cell wall of *Candida albicans* [25]. This technique, as well as the use of ^{99m}Tc-labeled fluconazole, the ^{18}F-labeled fluconazole and the ^{123}I-labeled chitinase may prove useful in distinguishing fungal from bacterial infections, but the data are still limited in animal studies [25,26]. There are human studies regarding the diagnostic value of PET with [^{18}F]fluorodeoxyglucose in invasive fungal infections, but the data on brain mycoses are still scarce [27].

Cerebrospinal fluid analysis

The characteristics of the cerebrospinal fluid (CSF) in cases of fungal meningitis are shown in Table 3. Cell count may be lymphocytic in cryptococcal disease and *Candida* meningitis, neutrophilic early in aspergillosis and blastomycosis or eosinophilic in coccidial meningitis [1,28]. In cases of neurosurgery-associated fungal meningitis, pleocytosis as well as other signs of infection may be absent [1]. The elevated opening pressure is associated with poor prognosis in cryptococcal meningitis, but it can also be elevated in cases of CNS blastomycosis or coccidioidomycosis [28,29].

The detection of galactomannan (GM) has not been validated for use in the CSF and the diagnosis of cerebral aspergillosis [12], while detection of (1-3)-β-D-glucan in the CSF might be useful for the diagnosis of different cerebral invasive fungal infections, including aspergillosis, histoplasmosis and probably cryptococcosis, but for both assays, data are limited [30]. PCR-based assays are not well validated for application on CSF samples in the diagnosis of cerebral mycoses. Some very limited studies report sensitivity up to 100% in cases of invasive aspergillosis [31]. Similar techniques have been developed for *C. immitis* [32], but they are neither validated nor widely available. Brain biopsy is often needed for the diagnosis of parenchymal disease, when conventional methods fail to reveal the pathogen, nevertheless such a procedure should always be weighed against empirical antifungal therapy [1,12]. Especially, image-guided stereotactic brain biopsies is associated with high diagnostic yield [8].

Specific pathogens & the CNS infections

Molds

Aspergillus spp.

Most cases of CNS aspergillosis involve the brain parenchyma, while cases of *Aspergillus* spp. meningitis are very rare [22,33]. Overall, intracranial spread occurs in 10–20% as a result of hematogenous dissemination and occasionally from direct extension [22]. Except for the classical predisposing factors mentioned above, diabetes, underlying brain pathology, trauma and introduction of the pathogen into CNS during a neurosurgical procedure have been proposed as risk factors [9]. Recent data suggest that certain receptor deficiencies (such as in Toll-like receptors or in dectin-1) may predispose to aspergillosis, but this research has not been specifically confirmed for CNS disease [34–36].

Aspergillosis involving the brain parenchyma is characterized by cerebral infarction that is followed by conversion to an infected area with abscess formation [22]. Involvement of the basal ganglia is characteristic [21,22] while, in the appropriate clinical scenario, the hyperintense lesions on diffuse weighted

doi: 10.1586/14787210.2014.874282

Table 3. Cerebrospinal fluid findings in fungal meningitis.

Pathogen	Cells	Protein	Glucose
Cryptococcus spp.	N or ↑	N or ↑↑↑	N or ↓
Penicillium marneffei	N or ↑	N or ↑	N or ↓
Histoplasma capsulatum	↑	↑	↓
Coccidioides immitis	↑	↑	↓
Paracoccidioides brasiliensis	↑	↑	N or ↓
Exserohilum rostratum	↑↑↑	N	N
Blastomyces dermatitidis	↑	↑	↓

N: Normal.

imaging may provide justification for prompt treatment initiation [22]. Image-guided stereotactic brain biopsy is associated with a diagnostic yield that for focal lesions can be as high as 88–90% [8].

As noted above, GM test has been validated only in blood and bronchoalveolar lavage specimens. Nevertheless, in cases where a brain biopsy cannot be obtained, a CSF positive for GM may help ascertain the diagnosis [37]. In a study, the detection of serum (1-3)-β-D-glucan in the CSF was helpful for the diagnosis of aspergillosis, however, only three patients with cerebral aspergillosis were included [30]. The detection of *Aspergillus* spp. DNA in the CSF with the use of the nested PCR assay that has been validated for blood and bronchoalveolar lavage secretions has been investigated [31]. The assay detected DNA of *Aspergillus fumigatus* or *Aspergillus flavus* in all six patients with cerebral aspergillosis and prospective studies with a sufficient number of patients are needed [31].

Other molds

Diabetes ketoacidosis and immunosuppression (such as hematologic malignancy, solid organ transplantation, hematopoietic stem cell transplant and high-dose steroids) have been the classical risk factors for mucormycosis [15,38,39]. Neurosurgery can be a predisposing factor and isolated CNS disease has been associated with illegal drug use [40]. CNS mucormycosis can manifest in three distinct clinical forms: rhinocerebral (which is the most common form, around 70% of all the cases involving the CNS), disseminated disease with CNS involvement or as isolated cerebral disease [40]. Similar to aspergillosis, mucormycosis is characterized by cerebral infarction associated with arterial obstruction and arteritis [38]. Venous occlusion may also occur through embolization and local acidosis and CNS invasion. Cavernous sinus thrombosis has been reported, as well as acute or subacute infarction in the spinal cord [15,38,40]. Histology and culture are needed for a definite diagnosis, but culture is positive in <70% of the cases. Mucormycosis-specific PCR-based methods may be helpful for the correct fungus identification on tissue samples, but the molecular techniques are not widely used [39,40].

The genus *Scedosporium* consists of two important species: *Scedosporium apiospermum* (and its sexual state *Pseudoallescheria boydii*) and *Scedosporium prolificans*. The disseminated form of the disease may include meningitis, meningoencephalitis, solitary or multiple brain abscesses, vasculitis and occasionally true mycotic aneurysms and intracerebral hemorrhage [41]. Meningitis is a rare syndrome and it can be associated with immunosuppression but also CSF drainage devices and rachianesthesia. Its evolution is rapid and it may coexist with abscess formation and infarcts [41]. Invasion of the CNS may occur through hematogenous dissemination, through the paranasal sinuses, the cribiform plate and through unapparent skull fractures [41]. There is a distinctive clinical syndrome caused by *P. boydii* occurring in immunocompetent individuals suffering near drowning in polluted waters [41]. This clinical syndrome usually presents within days or weeks after the near-drowning incident, but delays up to 4 months have been reported. Imaging includes single or multiple brain abscesses [41].

Primary cerebral phaeohyphomycosis in humans is increasingly recognized as an infectious disease associated with poor prognosis [42]. The main neurotropic agents include *Cladophialophora bantiana*, *Wangiella dermatitidis*, *Chaetomium atrobrunneum* and *Rhinocladiella mackenziei* [43]. The genus *Cladophialophora* represents around 50% of the isolated organisms of the cases of primary cerebral phaeohyphomycosis [42,44]. Infection by *R. mackenziei* targets exclusively the brain. This infection is thought to be restricted to the Middle East (with an unknown natural niche). Both immunocompetent and immunocompromised individuals can be infected and the outcome is usually dismal [43].

Yeasts

Candida spp.

Involvement of the parenchymal brain tissue and the meninges can rarely result as a complication of hematogenously disseminated disease or neurosurgical procedures. Autopsy studies report that as many as 1–6% of patients with systemic disease also have CNS involvement [1,12] and *C. albicans* is the most prevalent pathogen [12]. CNS disease may present as cerebral micro-abscesses, abscesses, meningitis or vascular complications such as infarcts and subarachnoid hemorrhage [1]. Meningitis is rare and comprises <15% of the CNS candidiasis cases [1]. Detection of the *Candida* spp. antigen mannan in the CSF may be useful in the diagnosis of meningitis but data are limited [45].

Cryptococcus spp. & the emergence of *Cryptococcus gattii*

In North America, there are approximately 7800 cases of cryptococcal meningitis or meningoencephalitis per year with about 700 deaths, while in sub-Saharan Africa there are 720,000 cases per year with 504,000 estimated deaths [3]. The incidence of disease in HIV is 0.2/1000 person-years [8]. Cryptococcal infection is also seen in cancer patients (18 cases per 100,000 admissions) and in bone marrow transplant recipients [4], whereas in solid organ transplants, the incidence of cryptococcal CNS disease is 2.8% [1]. *Cryptococcus* spp. use a forceful way to

overcome the blood–brain barrier through an invasion of the monocytes [46,47].

Until the outbreak in Vancouver Island, British Columbia, *C. gattii* infection was thought not to occur in temperate climates [47–50]. Between 1999 and 2007, 218 cases were reported, which represents an annual incidence of 5.8 cases per million residents per year [48,49,51] with a peak of 27 per million residents between 2002 and 2006 [49,51,52]. In the analysis of the outbreak, 7.8% of the patients presented with a CNS syndrome and 10.1% with both a respiratory and a CNS syndrome [51]. *C. gattii* infection frequently occurs in hosts with seemingly intact immune systems, a fact that warrants increased awareness [4,52]. While these patients may represent a heterogeneous group, a maladapted immune response to cryptococcal exposure may allow for the progression to clinical cryptococcal disease [3,49,53]. The *C. gattii* outbreak has been extensively covered in the literature [49,51,54].

In the HIV patient, cryptococcal meningitis can be the first presentation of the disease, typically occurring when the CD4 cell count is <50 cells/μl [8]. In patients in whom cART is initiated, immune reconstitution inflammatory syndrome might reveal a latent CNS infection [8]. Immune reconstitution inflammatory syndrome is especially common within the first 3 months after cART commencement. It has been reported in up to 30.5% of AIDS patients who started cART following treatment with culture-negative meningitis being the presentation [4,55]. The reconstitution syndrome has also been reported in organ transplant recipients [56].

Initial CSF analysis may not show pleocytosis, hyperproteinorrachia or hypoglycorrhachia in about 30% of the cases; therefore, repeat analyses may be needed [8]. Culture and staining (with India ink, calcofluor white or other stains) of CSF as well as cryptococcal polysaccharide antigen detection in the serum and CSF comprise the traditional diagnostic techniques [3,8,29]. The India ink method is highly sensitive (>90%) and specific (approaching 100%) in the hands of experienced technicians, nevertheless the sensitivity is lower when performed in non-HIV-positive individuals [8,29]. Some modern laboratories use calcofluor white staining but the procedure requires a fluorescence microscope [57]. In some patient populations, serum cryptococcal antigen titer is positive in approximately 85–90% [29,58]. In CSF, cryptococcal antigen positivity approaches 100%. It is measured using a latex-agglutination or an enzyme immunoassay kit [29]. The test performs very well regardless of the different hosts (immunocompetent, AIDS patients, immunocompromised/non-HIV-positive, organ transplant recipients) [58]. Lateral flow immunoassay is a marketed new, inexpensive and rapid diagnostic method with comparable results with the enzyme immunoassay for cryptococcal antigen and can be used on whole blood or urine as a dipstick test [29,59].

Trichosporon spp.

CNS disease due to *Trichosporon* spp. is rare but persistent infections that cause disseminated disease may allow the fungus to establish in sterile sites such as the brain [60]. The invasive properties of capsular antigen glucuronoxylomannan may account for the meningeal involvement in the setting of a breached blood–brain barrier and enables the fungus to evade phagocytosis [60].

Endemic fungi

Brain blastomycosis is an uncommon and potentially fatal complication of the disease and accounts for 5–22% of extrapulmonary disease [61,62]. In CNS infection, the cerebellum is frequently involved, while concomitant meningitis can be present. Other manifestations may include intracranial masses that may resemble meningiomas and abscesses of the spinal cord or epidural space. As CSF culture is insensitive for the diagnosis, detection of *Blastomyces dermatitidis* from histopathological specimens and tissue cultures are more useful [61,62].

Isolated CNS histoplasmosis is rare but it can happen in 5–10% of disseminated disease and is more common among immunosuppressed individuals [63]. The estimated burden of CNS disease is 1 per 100,000–500,000 infected people per year. The most commonly presentations of CNS histoplasmosis include chronic meningitis and histoplasmomas, whereas stroke syndromes and encephalitis are uncommon [64]. The infection may be latent for several years so it should be considered in patients who have lived in endemic areas [63,65]. Despite the fact that histoplasmosis may be the cause of the hydrocephalus, there have been several reports in people with a known cause of hydrocephalus who have developed a shunt infection with *H. capsulatum* [18]. A specimen of 10 ml may be required for a positive culture [64]. Detection of antibodies in the CSF is positive in 80% of the cases. However, cross-reaction with other yeasts is of concern. In cases where disseminated disease including CNS is suspected, antigen and antibody testing of blood and urine may be useful [64]. Eventually, biopsy may be required for the diagnosis [63,64].

Approximately 25% of the disseminated coccidioidomycosis cases have involvement of the CNS [66]. Dissemination of *C. immitis* to the CNS is more common among immunocompromised individuals (especially those who are HIV-positive or receive long-term immunosuppressive therapy), pregnant women and people of African or Asian descent [20,67,68]. The disease classically involves the basilar meninges and headache is a characteristic presenting symptom in 75% of the cases [20,68]. Diverse processes have been described, including abscesses, caseous lesions and small granulomas [66]. Focal brain lesions usually involve the posterior fossa [68] and cerebral infarction and thrombosis may be present [20]. CSF analysis classically reveals eosinophilia in 10–70% of the cases [20,68] and identification of *C. immitis* spherules or a positive CSF culture are diagnostic [20]. Unfortunately, CSF cultures are positive in only 15–20% of the cases [20,68], whereas biopsy and histopathology may provide the final diagnosis in cases of focal lesions [68]. Antibody detection in the CSF using a complement fixation method provided the most sensitive test for the diagnosis of meningitis but the sensitivity rates ranged from 48 to 91% in different series [20,68].

doi: 10.1586/14787210.2014.874282

PCR-based techniques are both sensitive and specific and may prove promising for the diagnosis of the disease since they provide a much faster result as compared with culture or serology [8,66].

Almost 10 million people are infected by *Paracoccidioides brasiliensis* and up to 2% of them may present with disseminated disease [69]. The prevalence of CNS disease ranges from 9.65 to 25.45% in different series. The male:female ratio is 23/1 for women in the reproductive age, which probably indicates an inhibitory action of estrogens on the transformation to the yeast form [69]. The granulomatous form of the disease predominates (96% of the cases) and meningitis may co-exist in 17% of the cases. Direct CSF examination and culture is usually normal. Brain hemispheres are most commonly affected (67%) [69]. The detection of an antibody to the gp43 of the yeast in the serum, urine or CSF may help in the diagnosis, but the CSF assay may also monitor the evolution of the disease [69].

Selected *Penicillium* spp. such as *Penicillium marneffei*, *Penicillium commune* and *Penicillium chrysogenum* have been isolated from the CSF and autopsy specimens of immunosuppressed individuals [70]. The involvement presents as an acute disease and there is only 50% co-existence with the typical *P. marneffei* skin lesions. CSF analysis ranges from acellular to mild pleocytosis with normal to mildly elevated protein level and low to normal glucose level. CSF culture can be positive but it may take up to 4 days [70]. Recently, a nested PCR method was evaluated for the identification of the pathogen in paraffin-embedded tissues and it may prove of assistance in diagnosis when tissue specimens are available [71].

Iatrogenic fungal meningitis outbreak 2012

In the late 2012, an unprecedented outbreak of fungal infections was identified in patients who received epidural steroid injections that were contaminated with environmental molds. The steroids used were all from the same batch of preservative-free solutions of methylprednisolone and the disease resulted from the direct inoculation of fungi into human tissue [7,72–75]. The overall case rate was near 4% of more than 13,500 exposed individuals, a fact that is probably due to host factors such as polymorphisms in immune molecules [72,76]. The outbreak eventually extended in over 20 states and as of 1 July 2013, the total case count stood at 749 with 61 deaths [72,74,75].

Although the index case was caused by *A. fumigatus*, subsequent reports indicated that the majority of affected patients were diagnosed with meningitis caused by *Exserohilum rostratum*, a black mold which is a plant pathogen and an extremely rare cause of human fungal disease [7,77,78]. Three primary syndromes were reported: meningitis or arachnoiditis alone (52 and 15%, respectively), cauda equina syndrome or focal infections/epidural abscesses with or without meningitis (43%) and posterior stroke (10%) [7,72,78–80]. Fungal invasion of brain parenchyma was infrequent. The pathophysiological characteristics of fungal meningitis in the outbreak resemble other CNS fungal infections, with fungal angioinvasion and vasculitis resulting in thrombosis and infarction [79,81]. The presentation of the disease as meningitis, probably suggests a synergistic combination of previously unrecognized neurotropism of the fungus and local CNS immunosuppression [7].

CSF analysis was notable for pleocytosis, with an average neutrophil count of 648 cells/mm^3 (white cell counts ranging 13–15,000 cells/mm^3 with a neutrophil predominance), suggesting that the inflammatory response may have contributed to host damage [7,75,80]. In most cases, there was no hyperproteinorrhachia or hypoglycorrhachia [78]. The fungus was identified from tissue and CSF samples, as well as the vials of the implicated lots of methylprednisolone by culture and DNA sequencing when the organism started to sporulate [73,76,77,82]. Culture from clinical samples, especially CSF was often negative due to the paucity of free-floating organisms. A rapid real-time PCR test was used to reliably and quantitatively identify *E. rostratum*. This enabled the diagnosis in the outbreak investigation, characterization and control and was also used for monitoring the therapeutic response [73,75,77,82]. Immunohistochemistry on formalin-fixed, paraffin-embedded tissues was highly sensitive for detection of the fungi in tissues [81]. Eventually, a microbiologic diagnosis was established in only 35% of the cases and *E. rostratum* was isolated in the majority. This low percentage of culture positivity could have been due to inappropriate sample collection [72,76]. Other organisms isolated included *Cladosporium cladosporioides*, *Aspergillus terreus*, *Alternaria alternate* and *Chaetonium* spp. [72].

Expert commentary

In the last decade, we have seen some very interesting developments in the prevalence of cerebral mycoses. We have witnessed the expansion of the patient populations that are susceptible to CNS mycoses to include individuals with apparently intact immune systems, patients who suffer from autoimmune diseases, and especially SLE as well patients who receive anti-TNF-α inhibitors.

Moreover, we have witnessed the expansion of fungal pathogens that adapt to new climatic niches or crossover from the plant and animal kingdoms to humans and cause new forms of CNS involvement. The former was epitomized by the *C. gattii* outbreak, a pathogen traditionally encountered in Australia and New Zealand, in Canada and the USA. The latter is typically represented by the large meningitis outbreak of 'invasive exserohilosis' that astonished the medical community.

While new pathogens emerge in new, unusual areas, endemic fungi remain significant causes of cerebral disease in immunosuppressed individuals including HIV-positive patients and patients suffering from autoimmune diseases. These pathogens should be included in the differential diagnosis of cerebral mycoses occurring in endemic areas, or in patients having lived in endemic areas since these fungi may remain dormant for several years.

In the era of geo-climatic changes and increasing numbers and complexity of invasive medical procedures, future outbreaks of unusual pathogens are not precluded. It is fundamental that

we develop early warning systems in order to contain future epidemics. Diagnostics play a major role in such containment and it is obvious that the future belongs to the improvement of 'non-culture' diagnostic procedures. Except for *Cryptococcus* spp. that may readily grow from CSF in cases of meningitis, the specimen culture for other type of fungi may be negative and a significant lag in positivity may exist. Molecular diagnostics with PCR-based techniques will prove invaluable in the future, especially since diagnostic procedures, such as brain biopsy, often cannot be performed in severely sick patients.

Five-year view

The main field of research regarding cerebral fungal infections lies in the development of new diagnostic procedures, with a special interest on molecular techniques in easily obtainable specimens such as CSF. This will minimize the need for invasive diagnostic procedures that are frequently non-feasible in the hosts susceptible to fungal mycoses. We also anticipate that in the next few years, we will encounter more cerebral mycoses in patients receiving newer immunosuppressive treatments, especially monoclonal antibodies for hematological malignancies and rheumatologic diseases.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending or royalties.

No writing assistance was utilized in the production of this manuscript.

Key issues

- Different patient groups have different susceptibility to specific fungi and understanding the different risk groups can assist in the differential diagnosis of cerebral mycoses.
- Knowledge of the local epidemiology and endemic nature of certain pathogens is important for diagnosis.
- New fungal pathogens cause human disease through a breach in the body's normal barriers, while the epidemiology of older fungal pathogens is also evolving.
- Antigen and antibody assays in the CSF are useful in diagnosing certain mycoses even when these include the brain parenchyma. Cryptococcal antigen CSF assay remains a traditional diagnostic tool. Histoplasma antigen and antibodies testing in the CSF have been well validated for diagnosis. Antibody detection is diagnostic for coccidioidal and paracoccidioidal disease.
- Molecular techniques are not validated for use in CSF samples in suspected cerebral mycoses.
- Imaging findings for cerebral mycoses are non-specific but newer techniques, such as diffusion-weighted imaging, magnetic resonance perfusion and magnetic resonance spectroscopy are helpful tools in the differential diagnosis of bacterial infections or tumors.

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doi: 10.1586/14787210.2014.874282